METHOD 3200

# MERCURY SPECIES FRACTIONATION AND QUANTIFICATION BY MICROWAVE ASSISTED EXTRACTION, SELECTIVE SOLVENT EXTRACTION AND/OR SOLID PHASE EXTRACTION

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

## 1.0 SCOPE AND APPLICATION

For a summary of changes in this version from the previous version, please see Appendix A at the end of this document.

1.1 This method contains a sequential extraction and separation procedure that may be used in conjunction with a determinative method to differentiate mercury species that are present in soils and sediments. This method provides information on both total mercury and various mercury species.

1.2 The speciation of a metal, in this case mercury, involves determining the actual form of the molecules or ions that are present in the sample. When combined with an appropriate determinative method, this procedure is designed to provide varying degrees of mercury species information. All metal speciation methods are operationally defined by the level of post-extraction processing and the chosen method of analysis.

The environmental mobility and toxicity of mercury in a soil profile depend on its speciation. Alkyl mercury species such as methylmercury are at least an order of magnitude more mobile than inorganic mercury species, and thus are more toxic and more readily bioaccumulated. Soluble inorganic mercury species such as mercury chloride are more easily transported by natural process than the other inorganic mercury species and serve as the substrate for mercury methylation process (Ref. 1). These extractable organomercury species and extractable inorganic species contribute the major portion of mercury potential toxicity in the soils. The mercury species that fall into the "semi-mobile" category such as elemental mercury are less toxic than extractable mercury species. The "non-mobile" mercury species such as mercury sulfide are chemically stable in the soil for geologic time periods and thus are least toxic.

Examples of the operationally-defined mercury fractions and individual species that may be determined using this procedure are presented in the table below.

| Operationally-Defined Mercury Fractions | | Individual Mercury Species | CAS No.[a] |
|---|---|---|---|
| Total Mercury | | | |
| Extractable Mercury | Extractable Organic Mercury | $CH_3HgCl$<br>$CH_3CH_2HgCl$ | 115-09-3<br>107-27-7 |
| | Extractable Inorganic Mercury | $HgCl_2$<br>$Hg(OH)_2$<br>$Hg(NO_3)_2$<br>$HgSO_4$<br>HgO<br>$Hg^{2+}$ complexes[c] | 7487-94-7<br>[b]—<br>10045-94-0<br>13766-44-4<br>21908-53-2<br>— |
| Non-extractable Mercury | Semi-mobile Mercury | $Hg^0$<br>$Hg^0$-M[d]<br>$Hg^{2+}$ complexes[c]<br>$Hg_2Cl_2$ (minor) | 7439-97-6<br>—<br>—<br>10112-91-1 |
| | Non-mobile Mercury | $Hg_2Cl_2$ (major)<br>HgS<br>HgSe | 10112-91-1<br>1344-48-5<br>20601-83-6 |

[a]Chemical Abstracts Service Registry Number
[b]Not registered by the Chemical Abstracts Service
[c]Certain inorganic mercury complexes may be present in both the organic and inorganic extractable fractions
[d]This represents a mercury-metal amalgam

1.3 Quantification of mercury in the different fractions may be performed using any suitable technique with appropriate precision and accuracy, for example Method 6800, 7473, 1631, or Methods 7470 and 7471. Other analytical techniques, such as gas chromatography/mass spectrometry (GC/MS), ion chromatography or high performance liquid chromatography (HPLC) with either GC/MS or inductively coupled plasma-mass spectrometry (ICP-MS) detection (Method 6020), or other hyphenated and/or mass spectrometric techniques, may be employed if performance appropriate for the intended application can be demonstrated. This method may also be applicable to other matrices, such as industrial and municipal waste materials, but its performance on such matrices has not yet been evaluated. Method 6800 (Elemental and Molecular Speciated Isotope Dilution Mass Spectrometry) may also be applicable as a diagnostic and validation tool for quantification of selectively extracted mercury species, especially when species transformations occur in the sample preparation or analysis procedures.

1.4 Prior to employing this method, analysts are advised to consult each method that may be employed in the overall analysis (e.g., Method 6800, 7473, 7470, or 7471) and the manufacturer's instructions for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a

regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.5 This method is restricted to use by, or under supervision of, personnel appropriately experienced and trained in the use of metal speciation and analysis techniques. Each analyst must demonstrate the ability to generate acceptable results with this method.

## 2.0 SUMMARY OF METHOD

2.1 For the determination of extractable mercury species, a representative sample aliquot is extracted with an appropriate volume of solvent at elevated temperatures. Extraction is accomplished with the aid of either microwave irradiation or ultrasound.

2.2 Following initial extraction the resultant extracts are separated from the remaining sample matrix for analysis of extractable mercury by an appropriate technique. The residual sample matrix may be analyzed for non-extractable mercury using an appropriate technique.

2.3 The method also has provisions for the separation of the extractable mercury fraction into inorganic and organic mercury fractions or individual species. The inorganic and organic mercury fractions may be separated by using a solid-phase extraction procedure. Individual species may be separated and determined by using an HPLC or other appropriate separation device coupled to an appropriate detector.

2.4 The method also has provisions for the separation of the non-extractable mercury fraction into semi-mobile and non-mobile mercury fractions using sequential acid extraction and digestion.

## 3.0 DEFINITIONS

3.1 Species - The actual form of a molecule or ion that is present in a sample. Ref. 2 contains additional definitions for “species.”

3.2 Sub-speciation - The process by which mercury species in the extractable mercury or non-extractable fraction are further subdivided. Ref. 2 contains additional definitions for “sub-speciation.”

3.3 Total mercury - Total mercury content within the sample, including all inorganic, organic, and complex forms of mercury.

3.4 Extractable mercury - An operationally-defined fraction of mercury which can be extracted from the sample using the protocols described within this method. The extractable mercury is meant to represent both organic and inorganic forms of mercury which are more labile.

3.5 Extractable inorganic mercury -- An operationally-defined subset of the extractable mercury species, i.e., the fraction of inorganic mercury species which can be extracted from the sample using the protocols described within this method.

3.6 Extractable organic mercury - An operationally-defined subset of the extractable mercury species, i.e., the fraction of organic mercury species which can be extracted from the

sample using the protocols described within this method.

3.7 Non-extractable mercury - The operationally-defined fraction of mercury remaining in the sample after using the extraction protocols described within this method. The non-extractable mercury is meant to represent the least labile forms of mercury.

3.8 Semi-mobile mercury - An operationally-defined subset of the non-extractable mercury species, i.e., the fraction of mercury species which can be extracted from the sample using the mild-acid extraction protocol(s) described within.

3.9 Non-mobile mercury - An operationally-defined subset of the non-extractable mercury species, i.e., the fraction of mercury species which can be extracted from the sample using the harsh-acid extraction protocol(s) described within.

3.10 Refer to Chapter One, Chapter Three, and the manufacturer's instructions for other definitions that may be relevant to this procedure.

## 4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Three for general guidance on the cleaning of glassware.

4.2 Transformations among mercury species have been reported and experimentally verified. For example, methylmercury formed during sample processing from inorganic mercury, may cause positive biases in the methylmercury results (Ref. 3 through 5). Also, a conversion of methylmercury and ethylmercury to inorganic mercury has been observed under certain sample processing conditions (Figures 1 and 2) (Ref. 6).

4.3 The possibility of species interconversions cannot be eliminated due to the necessary reagents, sample matrix, the combination of reagents and matrix, and/or the extraction method used. Method 6800 has successfully been used to monitor and correct for such species transformations during speciation of chromium and mercury (Ref. 7-9).

4.4 When non-specific detection techniques are employed, the analyst must be aware of possible interferences. For example, certain organic compounds may absorb at the wavelength of interest when ultraviolet (UV) detection is used.

NOTE: The adjustment of the pH during certain sample processing steps may result in the formation of a precipitate. Filtration (10-µm pore size or less) may be applicable to remove such a precipitate. Low recoveries may result from co-precipitation of mercury and other sample components. Rinsing the precipitate on the filter with a 0.1% HCl solution has been demonstrated to minimize this problem.

## 5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A

reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.2 The proper handling of inorganic and organomercury compounds cannot be overemphasized. Exposure to organo (alkyl) mercury compounds may cause damage to the central nervous system, emotional disturbances, or irritation of the eyes and skin, and may even lead to death (Ref. 10, 11).

5.3 The use of commercially-available protective gear, such as gloves made of nitrile, polyethylene (PE) and ethylvinyl alcohol (EVA) laminate or other appropriate material is required. Latex gloves are not suitable for the handling of organomercury compounds (Ref. 11) and *must* not be used. In addition, appropriate eye protection should be used.

5.4 If any organomercury compound makes direct contact with the gloves, remove the gloves immediately, dispose of them properly, and put on new gloves immediately. These materials only provide temporary protection. Consult the glove manufacturer for permeation rates and times. If contact should occur with the skin or eyes, flush with large amounts of water and seek medical attention immediately. Further information on the safety guidelines on the handling of inorganic and organo (alkyl) mercury can be obtained from the material safety data sheets for the substances.

5.5 The preparation and use of concentrated solutions or samples should be carried out in a fume hood *(some organomercury compounds are odorless)*. It is advisable to work inside a plastic container with absorbent pads so that accidental spills will be contained.

5.6 In the event of a spill, the area should be well ventilated and any ignition sources removed. If the spill is a solid, collect and dispose of the material in a sealed container. If the spill is a liquid, absorb on paper towels and discard in a designated waste bin. The contaminated area can also be cleaned with 0.05% (v/v) 2-mercaptoethanol solution to complex and remove the spilled mercury.

5.7 The extraction process may generate a moderate amount of pressure. Vessels used for extraction should be capable of withstanding these pressures or have pressure relief mechanisms or should be vented during the extraction process.

5.8 For safety precautions associated with the determinative methods, consult those methods directly.

## 6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list all common laboratory glassware (e.g., beakers and flasks) that might be used.

6.1 Analytical balance - Accurate to 0.01 g

6.2 Vials/bottles, amber glass - Sizes as appropriate, e.g., 20-mL, with PTFE-lined screw-caps or crimp-tops for storage of extracts.

6.3 Heating sources - Non-sonicating heating sources equipped with adjustable heating control able to maintain a temperature of 95 ± 2 °C (e.g., microwave heat unit, hot block, hot water bath or other equivalent)

6.4 Sonication heating source - Bath or horn type

6.5 Graduated cylinder or equivalent volume measuring device

6.6 Volumetric flasks - Sizes as appropriate

6.7 pH measuring device - Universal pH paper or calibrated pH meter

6.8 Solid-phase extraction system - Visiprep solid-phase extraction manifold (Supelco or equivalent system). Consult the manufacturer's recommendations for the glassware and hardware necessary to perform sample extractions.

6.9 Solid-phase extraction column - 6-mL glass reaction tubes (Supelco) or equivalent, complete with PTFE frits (2 per tube)

6.10 Filtration device - Vacuum or manual

6.11 Filters - The filter media should have an effective pore size of 1.0 µm or less (glass fiber filters are known to work effectively).

6.12 Temperature measurement device - Device should be capable of measuring up to 100 °C accurate to ± 0.1 °C.

6.13 Vortex mixer or equivalent

6.14 Centrifuge - Maximum speed of at least 3200 rpm and capable of handling 10-mL or greater centrifuge tubes

6.15 Centrifuge tubes - Disposable glass centrifuge tubes with capacity of at least 10 mL with snap-on cap

6.16 Microwave solvent extraction apparatus

6.16.1 The temperature performance requirements necessitate that the laboratory microwave extraction system be capable of sensing the temperature to within ± 2.5 ºC and automatically adjusting the microwave field output power within 2 sec of sensing. Temperature sensors should be accurate to ± 2 ºC. Temperature feedback control provides the primary performance mechanism for the method. Measurement in at least one vessel is required and a rotating turntable to homogenize the microwave field to all samples is required for most systems.

6.16.2 Microwave extraction vessels are needed. Vessels are available that can accommodate 1-g to 10-g samples. In addition the vessel apparatus must accommodate the necessary amount of solvent and if appropriate an inner vessel and stir bar and secondary microwave energy absorber. Vessels should be essentially transparent to microwave energy (with the exception of purposeful microwave absorbing apparatus components), relatively inert to reagents and sample components, and capable of

withstanding the temperature and pressure requirements (minimum conditions of 200 °C and 442 psi) necessary to perform this procedure. Follow the manufacturer's instructions regarding cleaning, handling, and sealing the vessels (Ref. 12).

## 7.0 REAGENTS AND STANDARDS

Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.1 Hydrochloric acid (concentrated, 12 M), HCl - Certified ACS Plus grade or equivalent

7.2 Sodium hydroxide, NaOH - Certified ACS grade or equivalent

7.3 Sodium chloride, NaCl - Certified ACS grade or equivalent

7.4 Copper (II) chloride dihydrate, $CuCl_2 \cdot 2H_2O$ - Analytical reagent grade or equivalent

7.5 Nitric acid (concentrated, 16 M), $HNO_3$ - Certified ACS Plus grade or equivalent

7.6 Hydrochloric acid (6 M), HCl - Prepared by dilution of 12 M HCl in reagent water

7.7 Sodium hydroxide (10 M), NaOH - Prepare by dissolution of solid NaOH pellets of appropriate purity in reagent water

7.8 Ethanol, $CH_3CH_2OH$ - HPLC grade or equivalent

7.9 Silver nitrate, $AgNO_3$ - Certified ACS grade or equivalent

7.10 HPLC subspecies option mobile phase components

7.10.1 Methanol, $CH_3OH$ - HPLC grade or equivalent

7.10.2 2-Mercaptoethanol, $HSCH_2CH_2OH$ - reagent grade

7.10.3 Ammonium acetate, $NH_4CO_2CH_3$ - reagent grade

7.11 Acetic acid, $CH_3CO_2H$ - HPLC grade or equivalent

7.12 Sulphydryl cotton fiber (SCF) separation and concentration option

7.12.1 Materials for preparing SCF

7.12.1.1 Cotton - Mercury-free cotton fiber

7.12.1.2 Mercaptoacetic acid (97+%), $HSCH_2CO_2H$ - analytical reagent grade

7.12.1.3 Acetic anhydride, $(CH_3CO)_2O$ - HPLC grade or equivalent

7.12.1.4 Sulfuric acid, (concentrated, 18 M), $H_2SO_4$ - analytical reagent grade or spectrograde quality

7.12.2 SCF eluent solutions

7.12.2.1 SCF eluent 1, an aqueous solution containing 1.0 M HCl and 1.0 M NaCl -- Prepare by diluting 20.7 mL of concentrated HCl to 250 mL in reagent water, then dissolving 14.6 g of NaCl in the prepared 1.0 M HCl.

7.12.2.2 SCF eluent 2, an aqueous solution containing 6 M HCl, saturated NaCl, and 0.1% $CuCl_2 \cdot 2H_2O$ -- Prepare by diluting 124 mL of concentrated HCl to 250 mL in water, then adding 0.25 g of $CuCl_2 \cdot 2H_2O$ and 11.0 g of NaCl.

NOTE: A portion of the solid NaCl may remain un-dissolved and settle to the bottom of the vessel. The top portion of this solution should be used.

7.13 Reagent water - Reagent water must be interference free. All references to water in the method refer to reagent water unless otherwise specified.

7.14 2.0% (v/v) HCl + 10% (v/v) ethanol extraction solution - Prepared by dilution of the proper amount of concentrated HCl and ethanol in reagent water.

7.15 1:2 (v/v) $HNO_3$ extraction solution - Prepare by combining 1 part concentrated $HNO_3$ with 2 parts reagent water.

7.16 1:6:7 (v/v/v) HCl : $HNO_3$ : reagent water - Prepare by combining 1 part concentrated HCl, 6 parts concentrated $HNO_3$ with 7 parts reagent water

7.17 Chloride ion test solution - 0.1 M silver nitrate in 0.1 M $HNO_3$. Commercially prepared 0.1 M silver nitrate solution is available.

7.18 4 M $HNO_3$ extraction solution - Prepared by dilution of the proper amount of concentrated $HNO_3$ in reagent water.

7.19 Inorganic mercury standard solutions - Commercially-prepared standards containing natural isotopically-abundant inorganic mercury species and isotopically-enriched inorganic mercury species, such as $Hg^{2+}$, are available (Ref. 13, 14). Standard solutions can also be prepared by dissolution of the selected pure solid mercury compound in an appropriate solvent. Mercury compounds must be of known concentration. All solvents used for standards preparation must be analytical reagent grade or equivalent.

7.20 Organic mercury standard solutions - Commercially prepared standards containing natural isotopically abundant $CH_3HgCl$ or other organic mercury species and/or isotopically-enriched $CH_3HgCl$ or other organic mercury species are available (Ref. 13, 14). Standard solutions can also be prepared using the pure organic mercury compound and appropriate solvents (Ref. 4). Pure compounds must be of known concentration. Solvents used for standards preparation must be analytical reagent grade or equivalent.

7.21 Sodium acetate, anhydrous, $NaCH_3CO_2$ - Certified ACS grade or equivalent

7.22 Sodium acetate trihydrate, $NaCH_3CO_2 \cdot 3H_2O$ - Certified ACS grade or equivalent

7.23 0.2 M acetate buffer solution (pH 3.0) - Mix 11.4 mL of acetic acid and 0.2789 g of anhydrous sodium acetate or 0.4627 g of sodium acetate trihydrate in reagent water. Dilute to 1 L with reagent water. Measure the pH of the buffer solution and if needed adjust the pH with a strong acid or base.

## 8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation and storage requirements.

8.1 See the introductory material of Chapter Three, “Inorganic Analytes.”

8.2 Samples should be collected and placed in containers that are made of glass or other appropriate material.

8.3 Sample extracts should be analyzed within 5 days (Ref. 15). This unique holding time recommendation will be added to the Update V version of Chapter Three.

## 9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean reference matrix. This will include a combination of the sample extraction method and the determinative method (e.g., a 7000 series method). The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000 for information on how to accomplish a demonstration of proficiency.

9.3 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the analytical species of interest as a safeguard against chronic laboratory contamination. The blanks should be carried through all

stages of sample preparation and analysis. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

The laboratory should not subtract the results of the method blank from those of any associated samples. Such “blank subtraction” may lead to negative sample results. If the method blank results do not meet the project-specific acceptance criteria and reanalysis is not practical, then the data user should be provided with the sample results, the method blank results, and a discussion of the corrective actions undertaken by the laboratory.

9.4 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, method sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, a duplicate, a matrix spike and a laboratory control sample (LCS) in each analytical batch. Any method blanks, matrix spike samples, and replicate samples should be subjected to the same analytical procedures (Secs. 4.0 and 11.0) as those used on actual samples. An example of Quality Control is described in section 13.8 (ref 26), demonstrates a mass balance method for mercury species where the interchanges between mercury species are quantified by measuring each species and then correcting for the inter-species transformations with method 6800 and obtaining mass balance by thermally grouping remaining non-extractable species as a single mercury species. These species are added together and compared with total mercury values independently measured by methods 3200 and 6800. The species added together equal the total mercury to, within the confidence interval, total combined measurement uncertainty to achieve mass balance. Method 3200 should not be used with other analytical methods except 6800 if the mercury species are active and transform from one species to another as demonstrated in Reference 26 and Method 6800. Species spike recovery is also a method of demonstration of Species stability.

9.4.1 Method blank - For each analytical batch of samples processed, at least one method blank should be carried throughout the entire sample preparation and analytical process. A method blank is prepared using the same reagents and quantities used with samples and processed through the appropriate steps of the procedure with the samples. These steps may include, but are not limited to extraction, chromatographic separation, concentration, dilution, filtering, and analysis. If the method blank does not contain target analytes at a level that exceeds the project-specific criteria requirements, then the method blank would be considered acceptable. In the absence of project-specific criteria, if the blank is less than the lower limit of quantitation, less than 10% of the regulatory limit, or less than 10% of the lowest sample concentration, whichever is greater, then the method blank is considered acceptable. If the method blank cannot be considered acceptable, the method blank should be re-analyzed at once, and if still unacceptable, then all samples after the last acceptable method blank must be re-prepared and re-analyzed along with the other appropriate batch of QC samples. These blanks will be useful in determining if samples are being contaminated. If the method blank exceeds the criteria, but the samples are all either below the reporting level or below the applicable action level or other criteria, then the data should not be rejected based on this analysis.

9.4.2 Matrix spike (MS)/ Duplicate - For each batch of solid phase samples extracted using this method, at least one matrix spike (MS) and one duplicate unspiked sample or one matrix spike/matrix spike duplicate (MS/MSD) pair should be carried

through the entire sample extraction, preparation and analytical process. An MS is an intra-laboratory split sample spiked with a known concentration of each analytical species of interest (see Secs. 7.19 and 7.20). Spiking is performed directly on the solid sample matrix prior to extraction in order to assess the efficiency of the extraction procedure. The decision on whether to prepare and analyze duplicate samples or a MS/MSD must be based on knowledge of the samples in the sample batch. If samples are expected to contain the target analyte, laboratories may use a matrix spike (MS) and a duplicate analysis of an unspiked field sample. If samples are not expected to contain the target analyte, the laboratories should use a MS/MSD pair. A separate spiked sample and a separate duplicate unspiked sample may be analyzed in lieu of MS/MSD analyses. MS samples should be spiked at the project-specific action level or, when lacking project-specific action levels, between the low- and mid-level calibration standards. Acceptance criteria should be set at a laboratory-derived limit developed through the use of historical analyses per matrix type analyzed. In the absence of historical data, the accuracy limit should be set at ±25% of the spiked concentration for the MS and 25% RPD for the MS/MSD or duplicate precision. Matrix spike/duplicate or MS/MSD samples should be included whenever a new matrix type is being analyzed.

9.4.3 LCS - A laboratory control sample (LCS) should be included with each analytical batch of samples processed. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes, at the same concentrations, as the matrix spike, when appropriate. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results may be used to verify that the laboratory can perform the analysis in a clean matrix.

NOTE: A standard reference material (SRM), certified reference material (CRM) or reference material (RM) may be used in place of an LCS. The SRM, CRM or RM generally consists of a commercially-prepared, well characterized matrix similar to the sample matrix and containing the analytical species of interest at established reference concentration levels.

9.5 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

## 10.0 CALIBRATION AND STANDARDIZATION

The laboratory microwave system should be calibrated to measure within ±2 °C. Consult the manufacturer's instructions for microwave system calibration.

## 11.0 PROCEDURE

11.1 The specific procedures that are applied to the sample depend on the extent of the mercury speciation information that is required.

11.1.1 If only total mercury data are required, then analyze the sample using Method 7473 or other suitable procedures, or sum the results for the extractable and non-extractable mercury fractions from Sec. 11.8.

11.1.2 If extractable mercury data are required, then proceed to Sec. 11.2 for the mercury extraction procedures and use either the microwave-assisted extraction procedure (Sec. 11.2.1) or the ultrasound-assisted extraction procedure (Sec. 11.2.2).

11.1.3 If non-extractable mercury data are required, then proceed to Sec. 11.2 for the mercury extraction procedures, but analyze the residual solids from the extraction procedure as stated in Sec. 11.2.1.5 or 11.2.2.8.

11.1.4 If data are required for extractable organic mercury, extractable inorganic mercury, or both fractions, proceed to Sec. 11.2 for the extraction procedures and then to Sec. 11.4 for the separation and concentration of the extractable mercury by solid-phase extraction, followed by Sec. 11.5 for the sub-speciation of the extractable organic and extractable inorganic mercury fractions.

11.1.5 If data for individual extractable mercury species are required, proceed to Sec. 11.2 for the extraction procedures and then proceed to Sec. 11.6 for the sub-speciation of the individual extractable mercury species by HPLC or appropriate hyphenated technique (see Sec. 11.8).

11.1.6 If data are required for semi-mobile mercury, non-mobile mercury, or both fractions, proceed to Sec. 11.2 for the mercury extraction procedures. Then, process the residual solids generated in Sec. 11.2 through the separation and extraction procedures in Sec. 11.7 for the sub-speciation of the semi-mobile and non-mobile mercury.

11.1.7 For some projects, it may be appropriate or required to report the results of the mercury speciation analyses relative to the dry weight of the original sample. The calculation of the dry weight of the sample is described in Sec. 12.1.

11.2 Extractable mercury

11.2.1 Microwave-assisted extraction

This extraction involves the use of a solution of 4.0 M $HNO_3$ to extract mercury species from soil or sediment samples (Ref. 16-18).

11.2.1.1 Weigh approximately 1 g ($\pm$ 0.2 grams, to at least 3 significant figures) of homogenized soil or sediment sample into microwave extraction vessels.

11.2.1.2 Add 10.0 mL of 4.0 M $HNO_3$ to each sample. Add a magnetic stirring bar to each vessel for thorough mixing of solvent with the sample.

11.2.1.3 Seal microwave vessels and irradiate at 100 °C for 10 min with magnetic stirring on. A 2-min ramping time should be used to reach the desired temperature of 100 °C.

NOTE: Longer ramping time may be used depending on the microwave power loading of the system. The extraction time of 10 min should be maintained independently of the ramping time.

11.2.1.4 Let the vessels cool for safe handling, and then filter the extracts through a 0.22-µm glass fiber filter. Store extracts in the cold at $\leq$6 °C until analysis (within 5 days). This extract contains the extractable mercury species and can be analyzed directly, concentrated and then analyzed or divided

for further processing. See Sec. 11.8 for analysis, Sec. 11.4 for separation and concentration and Sec. 11.5 for further speciation.

11.2.1.5 The residual solids may be saved for the direct analysis of the non-extractable mercury by Method 7473 or may be further extracted as semi-mobile and non-mobile mercury species. See Sec. 11.8 for analysis and Sec. 11.3 and 11.7 for further speciation.

11.2.2 Ultrasound-assisted extraction

This extraction involves the use of a solution of 2.0% HCl + 10% ethanol to extract mercury species from solid or sediment samples.

11.2.2.1 Weigh approximately 1 g (± 0.5 g, to at least 3 significant figures) of sample into a centrifuge tube.

11.2.2.2 Add 2.5 mL of 2.0% HCl + 10% ethanol extraction solvent to each sample. Make sure each tube is properly sealed.

11.2.2.3 Vortex the samples for 1 min then centrifuge the samples for 1 min. Test the pH value of the supernatant. If the pH is greater than 3, add concentrated HCl drop-wise; then vortex, centrifuge, and again test the pH. Repeat this step until the pH value of the supernatant is between 1.5 and 3. It is recommended to add a few drops (1 drop = 0.05 mL; not more than 5 drops) of concentrated acetate buffer (0.2 M, pH 3.0) to the supernatant, before adding HCl, to achieve this pH with minimal reagent addition.

11.2.2.4 Vortex the samples for 1 min. Sonicate samples for 7 min at 60 ± 2 °C.

11.2.2.5 Centrifuge the samples for 5 min at 3100 ± 100 rpm. Decant the supernatant into an appropriate container.

11.2.2.6 Repeat the extraction (Secs. 11.2.2.2, 11.2.2.4 and 11.2.2.5) three more times. Additional pH adjustment (Sec. 11.2.2.3) is not necessary. Combine all collected extraction supernatants into the same vessel.

11.2.2.7 Add 2.5 mL of reagent water to the sample residue. Vortex the samples for 1 min, and then centrifuge the samples for 1 min. Combine the water rinse with extraction supernatants from 11.2.2.6. This combined solution contains the extractable mercury species and can be analyzed directly, concentrated and then analyzed, or divided for further processing. See Sec. 11.8 for analysis, Sec. 11.4 for separation and concentration and Sec. 11.5 for further speciation.

11.2.2.8 The residual solids may be saved for the direct analysis of the non-extractable mercury by Method 7473 or further extracted as semi-mobile mercury species and non-mobile mercury species. See Sec. 11.8 for analysis and Secs. 11.3 and 11.7 for further speciation.

11.3 Further speciation of extractable mercury and non-extractable mercury

Determine if the extractable mercury fraction is to be divided into organic and inorganic fractions or into individual mercury species. To concentrate the extractable mercury fraction

and/or separate it into inorganic and organic mercury fractions, proceed to Sec. 11.4. To separate the extractable mercury fraction into individual mercury species, proceed to Sec. 11.6. To separate the non-extractable mercury fraction into semi-mobile and non-mobile mercury fractions, proceed to Sec. 11.7.

Sub-speciation by alternative techniques, such as GC-MS or chelation resins, may also be appropriate, provided that adequate performance can be demonstrated.

11.4 Isolation of extractable mercury by solid-phase extraction

This procedure is appropriate for the separation and concentration of the extractable mercury fraction using solid-phase extraction. Adequate separation has been achieved using SCF as the solid-phase extraction medium (Ref. 6, 15, 16, 19-21). The medium is commercially available (Ref. 13) or can be prepared in house following the procedure described below.

11.4.1 Prepare a mixture containing 50 mL of mercaptoacetic acid, 35 mL of acetic anhydride, 16 mL of acetic acid, 0.15 mL of sulfuric acid and 5 mL of reagent water in a clean vessel. Immerse a 15-g portion of cotton fiber in this mixture. Cover the vessel and place it in a constant temperature apparatus capable of maintaining a temperature of 40 ± 2 °C for four days. Remove the cotton fiber from the reagent mixture and place it in a filter funnel (or alternative vacuum filtration device) and rinse with reagent water until the pH of the washings is neutral. Dry the cotton fiber (now "SCF") at 40 ± 2 °C for two days. Store the SCF in a cold location at ≤ 6 °C or in a refrigerator in a dark bottle wrapped with aluminum foil. The SCF product is stable for at least 3-4 months (Ref. 20). Alternatively, purchase SCF solid phase columns commercially.

11.4.2 Place a PTFE frit at the bottom of an appropriate size column. Add a 0.2-g portion of the SCF, along with 3 mL of water. Place a second PTFE frit on the top of the SCF. Apply pressure to the top frit to compact the SCF into a homogeneous disk between the two frits.

NOTE: The bed volume of the SCF disk is approximately 0.7 mL per 0.1 gram of SCF solid-phase material. For example, in a column with an internal diameter of 1.1 cm, the height of the disk is about 1.4 cm. Higher capacity can be achieved by using more SCF; however the reagents for separation and fractionation should be proportionally increased.

NOTE: The column should be packed on the same day that the separation procedure will be performed and the SCF disks should be prepared with care to avoid channeling. If using commercially available SCF disks, then same day packing is not required.

11.4.3 Just prior to use condition the SCF disk by passing 10 mL of reagent water, then 10 mL of 6 M HCl and finally 15 mL of reagent water sequentially through the medium using a flow rate of 1 mL/min.

NOTE: The conditioning procedure should be performed just before the separation. After conditioning, the SCF media must be used within 30 min.

11.4.4 Adjust the pH of the extract from Sec. 11.2.1.4 or Sec. 11.2.2.7 of the extraction procedure to pH = 6 ± 1 with 10 M NaOH. Filter the solution to retain the particles whose sizes are larger than 1.0 µm. Rinse the retained particles with 3 mL 0.1% HCl. Combine the filtered solution with the rinse solution.

NOTE: The presence of large amount of $Fe^{3+}$ in the extracted solution will degrade the retention and separation ability of the SCF media. It may also induce the transformation from alkyl mercury to inorganic mercury under acidic condition. After adjusting the pH to 6 ± 1, the precipitation of $Fe(OH)_3$ is achieved. 1.0-µm filtration will minimize the presence of $Fe^{3+}$ in the solution before it is passed through the SCF column. The 0.1% (v/v) HCl rinse will minimize the loss of Hg species of interest during the filtration procedure (Ref. 6).

NOTE: 10 M NaOH is preferred over concentrated $NH_4OH$ for pH adjustment because $NH_4OH$ may precipitate out $Hg^{2+}$.

11.4.5 Adjust the pH of the filtered solution to pH = 3 ± 1 with 6 M HCl and pass through the SCF column using a flow rate of ≤1 mL/min.

11.4.6 If only the extractable mercury fraction is of interest, remove the SCF disk from the column and analyze it directly using an appropriate determinative method such as Method 7473 (Ref. 6, 22) to determine the amount of extractable mercury.

11.4.7 Alternatively, the concentrated extractable mercury fraction can be further separated into extractable inorganic mercury and extractable organic mercury fractions. If such separation is needed, proceed to 11.5.

11.5 Sub-speciation of inorganic and organic extractable mercury

11.5.1 Elute the organic mercury species from the SCF solid phase extraction medium by passing 8 mL of eluent 1 (see Sec. 7.12.2.1) through the SCF column at a flow rate of ≤1 mL/min, followed by 2 mL of water. This eluent is analyzed using an appropriate determinative method to determine the amount of organomercury that has been extracted from the sample. See Sec. 11.8 for details.

11.5.2 The extractable inorganic mercury fraction will remain on the SCF and may be analyzed by an appropriate mercury detection method, either by direct analysis of the solid SCF medium or by appropriate dissolution of the medium and subsequent analysis. See Sec. 11.8 for details.

11.5.3 As another option, the remaining inorganic mercury fraction can also be eluted from the SCF medium by passing 8 mL of eluent 2 (see Sec. 7.12.2.2) through the SCF column at a flow rate of ≤1 mL/min, followed by 2 mL of water. This eluent is analyzed using an appropriate determinative method to determine the amount of inorganic mercury that has been extracted from the sample. See Sec. 11.8 for details.

11.6 Sub-speciation of extractable mercury by HPLC

This procedure is appropriate for the separation of the extractable mercury fraction into individual mercury species: $HgCl_2$, $Hg(OH)_2$, $Hg(NO_3)_2$, $HgSO_4$, HgO, $Hg^{2+}$ complexes as $Hg^{2+}$ ion, and $CH_3Hg^+$ and $CH_3CH_2Hg^+$ ions.

11.6.1 Adequate separation has been achieved by HPLC using a 30 cm x 4.0 mm, 5-µm pore size C-18 column and a mobile phase containing 30:70 methanol:$H_2O$, 0.005% 2-mercaptoethanol, 0.6 M ammonium acetate (Ref. 8, 9).

11.6.2 To ensure compatibility with the HPLC column, adjust the pH of the extract from either Sec. 11.2.1.4 or Sec. 11.2.2.7 or Sec. 11.5.1 or Sec. 11.5.3 to a value in the range of 3 to 7. Dilute samples to an appropriate volume using reagent water.

NOTE: Certain metals which may be extracted from the soil along with the mercury species may precipitate when the pH is raised.

11.6.3 A precipitate formation due to the pH adjustment step may interfere with the separation or the detection method, in which case it may be necessary to filter the sample. Refer to Sec. 11.4.4

11.6.4 If the presence of a large amount of inorganic mercury interferes or masks the organic mercury peak then the SCF separation described in Sec. 11.4 can be used to eliminate this problem.

11.6.5 Refer to Method 8000 for additional information on HPLC analysis, Method 6800 for speciated isotope dilution, and other appropriate analytical detection methods.

11.7 Sub-speciation of non-extractable mercury

If needed, the remaining matrix material from Sec. 11.2.1.5 or Sec. 11.2.2.8 may be further divided as semi-mobile and non-mobile mercury fractions.

Sub-speciation of non-extractable mercury by alternative techniques, such as Method 3052 or other acid leaching and digestion procedure (Ref. 12, 23-25), may also be appropriate.

11.7.1 Semi-mobile mercury species

11.7.1.1 Add 5 mL reagent water to the remaining sample portion from Sec. 11.2.1.5 or Sec. 11.2.2.8. Vortex the sample continuously for 1 min.

11.7.1.2 Sonicate the sample for 1 min at 60 ± 2 °C.

11.7.1.3 Centrifuge the mixture for 5 min at 3200 rpm. Transfer the supernatant to a container.

11.7.1.4 Test the supernatant for chloride ions by adding 2-3 drops of chloride ion test solution (Sec. 7.17). Formation of a white precipitate or white turbidity indicates the presence of chloride ions.

11.7.1.5 Repeat the steps in Secs. 11.7.1.1-11.7.1.4 until the decanted supernatant is free of chloride ions. After testing for the presence of the chloride ion the supernatant should be discarded.

NOTE: The presence of chloride ions in the soil sample portion may result in the extraction of both semi-mobile and non-mobile mercury species in the same extraction step.

11.7.1.6 Add 5.0 mL of 1:2 (v/v) $HNO_3$ extraction solution to the remaining soil sample portion from 11.7.1.3. Make sure each vessel is properly sealed. Vortex the sample for 1 min.

11.7.1.7 Heat the mixture for 20 min at 95 ± 2 °C in a heating device described in Sec. 6.3.

NOTE: Gas may evolve, therefore, the vessel should be capped loosely during heating, unless the vessel is pressurizable.

11.7.1.8 Centrifuge the extracted mixture for 5 min. Transfer the supernatant to an appropriate container.

11.7.1.9 Repeat the steps in Secs. 11.7.1.6-11.7.1.8 once more. Combine all collected extraction supernatants in the same vessel.

11.7.1.10 Add 5.0 mL of reagent water to the remaining soil sample portion. Vortex the sample for 1 min and then centrifuge for 5 min. Decant and combine the rinse water with the extract supernatants. This solution contains the semi-mobile mercury species and can be analyzed by an appropriate mercury detection method. See Sec. 11.8 for details.

11.7.1.11 The residual solids may be saved for the analysis of the non-mobile mercury species.

11.7.2 Non-mobile mercury species

11.7.2.1 Analyze the solids from 11.7.1.11 directly for non-mobile mercury species (Sec. 11.8).

11.7.2.2 Alternatively, perform the following extraction (Secs. 11.7.2.3-11.7.2.7) and analyze the extract by appropriate means (Sec. 11.8).

11.7.2.3 Add 5.0 mL of 1:6:7 (v/v) HCl:$HNO_3$:reagent water extraction solution (Sec. 7.11) to the residual soil sample portion from 11.7.1.11. Make sure each vessel is properly sealed. Vortex the sample for 1 min.

11.7.2.4 Heat the mixture for 20 min at 95 ± 2 °C in a heating device described in Sec. 6.3.

NOTE: Gas may evolve, therefore, the vessel should be capped loosely during the heating, unless the vessel is pressurizable.

11.7.2.5 Centrifuge the extracted mixture for 5 min. Transfer the supernatant to an appropriate container.

11.7.2.6 Repeat the steps in Secs. 11.7.2.3-11.7.2.5 once more. Combine all collected extraction supernatants into the same vessel.

11.7.2.7 Add 5.0 mL of reagent water to the remaining soil sample portion. Vortex the sample for 1 min and then centrifuge for 5 min. Decant and combine the rinse water with the extract supernatants from Sec. 11.7.2.6. This solution contains the non-mobile mercury species and can be analyzed by an appropriate mercury detection method. See Sec. 11.8 for details.

11.8 Analysis of specific mercury fractions

Many mercury detection techniques are appropriate detection techniques, such as Methods 7473, 1631, 7470, 7471, 6020 and 6800. Other mercury detection techniques may also be appropriate, provided that adequate performance can be demonstrated.

11.8.1 Extractable mercury -- Analyze the extractable mercury fraction from Sec. 11.2.1.4, 11.2.2.7 or 11.5.3, using an appropriate detection technique.

NOTE: Pre-concentration may be necessary in some cases. The SCF solid-phase extraction procedure in Sec. 11.4 can be used for this purpose.

11.8.2 Extractable organic mercury -- Analyze the eluent from Sec. 11.5.1 by an appropriate detection technique. Alternatively, sum the individual organic species determined in Sec. 11.6.

11.8.3 Extractable inorganic mercury -- Analyze the SCF disk from Sec. 11.5.2 directly by Method 7473. Alternatively, analyze the eluent from Sec. 11.5.3 by an appropriate analytical detection technique. As another alternative, analyze either of the sample aliquots from Sec. 11.2.1.4, 11.2.2.7 or 11.5.3 by quantitating the inorganic peak as described in Sec. 11.6.

11.8.4 Non-extractable mercury -- Analyze the remaining solids from Sec. 11.2.1.5 or 11.2.2.8 directly by Method 7473 or by suitable decomposition such as Method 3052 and appropriate analysis procedures. Alternatively, determine the non-extractable mercury as the difference between the total mercury concentration (Sec. 11.8.5) and the extractable mercury concentration (Sec. 11.8.1).

11.8.5 Total mercury -- Analyze the unaltered sample by Method 7473 or by suitable decomposition such as Method 3052 and appropriate analysis procedures. Alternatively, sum the results from Secs. 11.8.1 and 11.8.4.

11.8.6 Semi-mobile mercury -- Analyze the extracted mercury fraction or a portion thereof from Sec. 11.7.1.10 using an appropriate detection technique.

11.8.7 Non-mobile mercury -- Analyze the remaining solids from Sec. 11.7.1.11 directly by Method 7473 or by other suitable decomposition such as Method 3052 and appropriate analysis procedures. Alternatively, analyze the extracted mercury fraction or a portion thereof from Sec. 11.7.2.7 using an appropriate detection technique (Ref. 12, 22).

## 12.0 DATA ANALYSIS AND CALCULATIONS

12.1 If appropriate for the specific project, calculate the sample dry weight fraction as follows:

$$\text{Dry Wt. Fraction} = \frac{W_2 - W_3}{W_1 - W_3}$$

where:

$W_1$ = Weight of sample + vessel before drying, in g

$W_2$ = Weight of sample + vessel after drying, in g

$W_3$ = Weight of empty, dry vessel, in g

12.2 The results of the analyses of the various mercury fractions can be converted to the concentration of the fraction in the solid sample as follows:

$$\text{Sample Concentration} = \frac{C \times V \times D}{W \times S}$$

where:

C = Concentration in the extract in mg/L
V = Volume of the extract in mL
D = Dilution factor from the analysis of the extract, if any
W = Wet weight of original sample aliquot that was extracted, in g
S = Dry-weight fraction of the sample, g/g (if dry-weight reporting is required)

The factors of 1000 in the numerator and the denominator convert mL to L and g to kg, effectively canceling each other out.

## 13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

13.2 The extractability of pure mercury species are summarized in Table 1 (Ref. 6). These data are provided for guidance purposes only.

13.3 The 2.0% HCl + 10% ethanol extraction procedure was evaluated by spiking natural matrices (mercury species spiked at 25 µg mercury per 1 g of sample). The natural matrices selected for spiking were silica ($SiO_2$), NIST SRM 2709 (San Joaquin Soil), and two soil matrices. The samples were analyzed by Method 7473 and HPLC-ICP-MS. The results are summarized in Tables 2 to 5. The "extractable" mercury fraction in 2.0% HCl + 10% ethanol extraction is compared with Method 1311 (TCLP) by using BCR 580 and SRM 2709. The results are summarized in Table 6. These data are provided for guidance purposes only.

13.4 The SCF solid-phase extraction was optimized. The optimal amount of SCF packed in column was determined to be 0.2 g with a bed volume of 0.7 mL/0.1 g SCF. The best pH range was 3 ± 1. The optimal flow rate was 1.0 mL/min. The optimal eluent 1 was a 1.0 M HCl + 1.0 M NaCl solution which minimizes the inorganic mercury interference on the organic mercury measurement. The optimal eluent 2 was a solution of 6 M HCl, saturated NaCl and 0.1% $CuCl_2 \cdot 2H_2O$. The overall performance of SCF solid-phase extraction procedure is summarized in Table 7. See Ref. 6 for details. These data are provided for guidance purposes only.

13.5 This method was validated with Institute for Reference Materials and Measurements (IRMM) reference material BCR 580. The results are summarized in Table 8. The samples were analyzed by EPA Method 7473 and HPLC-ICP-MS (Ref. 6). These data are provided for guidance purposes only.

13.6 The microwave-assisted extraction method was validated with two specifically prepared reference soil materials, three National Institute of Standards and Technology (NIST) standard reference materials (SRM 1941a, SRM 2704 and SRM 2709) and one IRMM reference material (BCR 580). The samples were analyzed by EPA Method 7473 and HPLC-ICP-MS. The results are summarized in Table 9 (Ref. 16, 17). These data are provided for guidance purposes only.

13.7 The microwave-assisted extraction method was also validated by using EPA Method 6800. These results indicate that, based on the matrices tested, the Method 3200 protocol does not induce any transformation from methylmercury to inorganic mercury or vice versa. The results are summarized in Table 10. See Ref. 16, 17. These data are provided for guidance purposes only.

13.8 The microwave-assisted extraction method was validated by mass balance, that is to say, all species were added up to equal the total mercury content in the samples by analysis of two crude oil samples using EPA Method 6800. The extractable and non-extractable fractions of mercury species were summed to calculate the total mercury content in those samples. Method 3200 was used to extract the extractable mercury species which consisted of inorganic, methyl-mercury and ethyl-mercury. The total mercury contents were determined by Method 6800 SIDMS after digestion with Method 3052. These results indicate that Method 3200 is suitable for mass balance study of mercury species. Non-extractable mercury was spiked in the extracted sample by Method 6800 IDMS and thermally converted to metallic mercury, then subsequently analyzed (Ref. 26). In addition, this research paper demonstrates that Method 3200, in combination with method 6800, facilitates measurement of mercury species' concentrations and including corrections of the interconversions among these mercury species and achieves mass balance of the mercury species. Mass balance is the independent measurement of total mercury and its comparison, by adding up all mercury species, a value that is compared and confirmed by the independent total mercury measurement. Table 11 and Reference 26 demonstrate that the mercury species in the sample equal the total, within the stated confidence interval, to the summed measurements; that is the 95% confidence interval in the case of the referenced research paper. The results are summarized in Table 11. These data are provided for guidance purposes only.

13.9 Both extraction protocols outlined in Method 3200 were demonstrated and validated by analyzing human hair reference material (IAEA-085). The results are summarized in Table 12 and Ref. 27. These results demonstrate that both extraction protocols are capable of extracting approximately 100% of several extractable mercury species from human hair, such as inorganic and methylmercury and that when Method 3200 is coupled with Method 6800 to correct for these species conversions, mass balance is achievable. These data are provided for guidance purposes only.

13.10 The microwave-assisted extraction method was demonstrated and validated by analyzing tuna fish reference material (ERM-CE-464). The tuna fish SRM was extracted and analyzed by using HPLC-ICP-MS. The percentage recovery of both mercury species was approximately 80% of the certified value. However, when the SRM was spiked with isotopically enriched analogues of mercury species and Method 6800 SIDMS is used for quantification, both mercury species were corrected to certified values. These data and those in related publications demonstrate that after equilibration (as described in Method 6800), the level of analyte recovery is no longer a factor affecting accuracy when Method 3200 is used in combination with Method 6800. The results are summarized in Table 13 (See Ref. 28). These data are provided for guidance purposes only.

## 14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety, http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

## 15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult the ACS publication listed in Sec. 14.2.

15.2 All inorganic mercury and organomercury waste should be stored separately in dedicated glass bottles. The waste bottles should be kept in a hood or ventilated area in containers equipped with absorbent pads. Gloves, pipette tips, etc., which might have come in contact with mercury containing compounds should be disposed in a dedicated waste receptacle fitted with a tight lid.

## REFERENCES


1. Bloom, N. S.; Gill, G. A.; Cappellino, S.; Dobbs, C.; McShea, L.; Driscoll, C.; Mason, R.; Rudd, J., "Speciation and Cycling of Mercury in Lavaca Bay, Texas, Sediments." *Environ. Sci. Technol.*, **1999,** 33, (1), 7-13.

2. Templeton, D.; Ariese, F.; Cornelis, R.; Danielsson, L. G.; Muntau, H.; van Leeuvan, H. P.; Lobinski, R., "Guidelines for terms related to chemical speciation and fractionation of elements: Definitions, structural aspects, and methodological approaches." *Pure Appl. Chem.*, **2000,** 72, (8), 1453-1470.

3. Hintelmann, H.; Falter, R.; Ilgen, G.; Evans R. D., "Determination of Artifactual Formation of Monomethylmercury ($CH_3Hg^+$) in Environmental Samples Using Stable $Hg^{2+}$ Isotopes with ICP-MS Detection: Calculation of Contents Applying Species Specific Isotope Additions." *Fresenius J. Anal. Chem.*, **1997,** 358, (3), 363-370.

4. Bloom, N. S.; Coleman, J. A.; Barber, L., "Artifact formation of methyl mercury during aqueous distillation and alternative techniques for the extraction of methyl mercury from environmental samples." *Fresenius J. Anal. Chem.*, **1997,** 358, (3), 371-377.

5. Rahman, G. M. M.; Kingston, H. M. S.; Bhandari, S., "Synthesis and Characterization of

Isotopically Enriched Methylmercury ($CH_3{}^{201}Hg^+$)." *Appl. Organomet. Chem.*, **2003,** 17, (12), 913-920.

6. Han, Y.; Kingston, H. M.; Boylan, H. M.; Rahman, G. M. M.; Shah, S.; Richter, R. C.; Link, D. D.; Bhandari, S., "Speciation of mercury in soil and sediment by selective solvent and acid extraction." *Anal. Bioanal. Chem.*, **2003,** 375, (3), 428-436.

7. Huo, D.; Kingston, H. M. S.; Larget, B., "Application of Isotope Dilution in Elemental Speciation: Speciated Isotope Dilution Mass Spectrometry (SIDMS)." In: Comprehensive Analytical Chemistry, Volume 33 (Elemental Speciation: New Approaches for Trace Element Analysis), Elsevier, New York, 2000; 277-313.

8. Rahman, G. M. M.; Kingston, H. M. S., "Application of Speciated Isotope Dilution Mass Spectrometry to Evaluate Extraction Methods for Determining Mercury Speciation in Soils and Sediments." *Anal. Chem.*, **2004,** 76, (13), 3548-3555.

9. Rahman, G. M. M.; Kingston, H. M. S.; Kern, J. C.; Hartwell, S. W.; Anderson, R. F.; Yang, S. -Y., "Inter-laboratory Validation of EPA Method 3200 for Mercury Speciation Analysis using Prepared Soil Reference Materials." *Appl. Organomet. Chem.*, **2005,** 19, 301-307.

10. Labor, U.S. Department of, Occupational Health Guideline for Organo (Alkyl) Mercury, Occupational Safety and Health Adminstration, Washington, D.C., 1978.

11. Nierenberg, D. W.; Nordgren, R. E.; Chang, M. B.; Siegler, R. W.; Blayney, M. B.; Hochberg, F.; Toribara, T. Y.; Cernichiari, E.; Clarkson, T., "Delayed Cerebellar Disease and Death after Accidental Exposure to Dimethylmercury." *New Engl. J. Med.*, **1993,** 338, 1672-1675.

12. Kingston, H. M.; Haswell, S. J., Microwave Enhanced Chemistry: Fundamentals, Sample Preparation, and Applications, 10th ed.; American Chemical Society, Washington, DC, 1997.

13. Applied Isotope Technologies, Inc., www.sidms.com, e-mail: info@sidms.com.

14. Kingston, H. M. Speciated Isotope Dilution Mass Spectrometry of Reactive Species and Related Methods. *US Patent 6 790 673 B1*, September 14, 2004.

15. Jain, W.; McLeod, C. W., "Rapid Sequential Determination of Inorganic Mercury and Methylmercury in Natural Waters by Flow-injection - Cold Vapor-Atomic-Fluorescence Spectrometry." *Talanta*, **1992,** 39, (11), 1537-1542.

16. Rahman, G. M. M.; Kingston, H. M. S., "Development of Microwave-Assisted Extraction Method and Isotopic Validation of Mercury Species in Soils and Sediments." *J. Anal. At. Spectrom.*, **2005,** 20, 183-191.

17. Rahman, G. M. M. "Development, Research and Validation of Environmental Speciation Methods: Evaluation by Speciated Isotope Dilution Mass Spectrometry in Mercury and Chromium Speciation Analysis." *Ph. D. Dissertation*, Duquesne University, Pittsburgh, PA, 2004.

18. Fischer, E.; Tu, Q.; Nagourney, S.; England, R.; Buckley, B. "Microwave-assisted Solvent Extraction for Quantitative Simultaneous Extraction of Inorganic Mercury and Methylmercury from Soils." *20th Annual National Environmental Monitoring Conference*, Washington, DC, July 19-23, 2004; Washington, DC, July 19-23, 2004.

19. Jain, W.; McLeod, C. W., "Field Sampling Technique for Mercury Speciation." *Anal. Proc.*, **1991**, 293-294.

20. Lee, Y. H.; Mowrer, J., "Determination of Methylmercury in Natural Waters at the Sub-nanograms-per-litre Level by Capillary Gas Chromatography after Adsorbent Pre-concentration." *Anal. Chim. Acta*, **1989,** 221, (2), 259-268.

21. Mena, M. L.; McLeod, C. W., "Mercury Species Immobilized on Sulphydryl Cotton: A New Candidate Reference Material for Mercury Speciation." *Mikrochim. Acta.*, **1996,** 123, (1-4), 103-108.

22. Boylan, H. M.; Richter, R. C.; Kingston, H. M.; Ricotta, A. C., "Rapid Analysis for the Field: Method Development and Application to Natural Gas Utility Sites." *Water Air Soil Poll.*, **2001,** 127, (1-4), 255-270.

23. Davis, A. Bloom, N. S.; Hee, Q.; Shane, S., "The Environmental Geochemistry and Bioaccessibility of Mercury in Soils and Sediments-A Review." *Risk Anal.*, **1997,** 17, (5), 105-113.

24. Revis, N. W.; Osborne, T. R.; Holdworth, G.; Hadden, C., "Distribution of Mercury Species in Soil from a Mercury-Contaminated Site." *Water Air Soil Poll.*, **1989,** 17, (5), 557-569.

25. Revis, N. W.; Osborne, T. R.; Sedgely, D.; King, A., "Quantitative Method for Determining the Concentration of Mercury (II) Sulphide in Soils and Sediments." *Analyst*, **1989,** 114, 823-826.

26. Rahman, G. M.; Fahrenholz, T. M.; Kingston, H. M. Skip; Pamuku, M.; Hwang, J. D.; Young, L.A., "Speciation of Mercury in Crude Oil uisng Speciated Isotope Dilution Mass Spectrometry." *Spectroscopy*, 2010, 25(1), 36-45.

27. Rahman, G.M.; Fahrenholz, T.M.; Kingston, H. M. Skip, "Application of Speciated Isotope Dilution Mass Spectrometry to Evaluate Methods for Efficiencies, Recoveries, and Quantification of Mercury Species Transformation in Human Hair." *J. Anal. At. Spectrom.,* 2009, 24, 83-92.

28. Reyes, L. H.; Rahman, G. M.; Fahrenholz, T.; Kingston, H. M. Skip, "Comparison of Methods with Respect to Efficiencies, Recoveries, and Quantitation of Mercury Species Interconversions in Food Demonstrated using Tuna Fish." *Anal. Bioanal. Chem.*, 20008, 390, 2123-2132.

## 17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

TABLE 1

EXTRACTABILITY OF MERCURY SPECIES

| | | Extractability (%) | | |
|---|---|---|---|---|
| Species* | Operationally-defined Mercury Fraction | 2.0% HCl + 10% Ethanol; 60 °C and sonication for 30 min | 1:2 $HNO_3$: reagent water; 95 °C for 40 min | 1:6:7 HCl:$HNO_3$: reagent water; 95 °C for 40 min |
| $CH_3HgCl$ | Extractable Organic | 98 | 99 | 104 |
| $C_2H_5HgCl$ | | 96 | 94 | 93 |
| $HgCl_2$ | Extractable Inorganic | 96 | 99 | 99 |
| HgO | | 97 | 97 | 102 |
| Hg | Semi-mobile | 4 | 95 | 102 |
| HgS | Non-mobile | 0.15 | 0.04 | 97 |
| $Hg_2Cl_2$ | | 1.6 | 11 | 96 |

*10 mg of each pure mercury species in each 10-mL extract solutions

Data obtained from Ref. 6.
These data are provided for guidance purposes only.

TABLE 2

RECOVERIES OF EXTRACTABLE MERCURY SPECIES SPIKED INTO A SILICA MATRIX

| Mercury spiked species | Recovery range* (%) HPLC-ICP-MS |
|---|---|
| $HgCl_2$ | $96 \pm 2$ |
| $CH_3HgCl$ | $98 \pm 6$ |
| $C_2H_5HgCl$ | $95 \pm 7$ |
| HgO | $98 \pm 4$ |

*95% confidence level, n = 3.

Data obtained from Ref. 6.
These data are provided for guidance purposes only.

TABLE 3

RECOVERIES OF EXTRACTABLE MERCURY SPECIES
SPIKED INTO SRM 2709 SOIL MATRIX

| Mercury spiked species | Recovery* (%) HPLC-ICP-MS | Recovery* (%) Method 7473 |
|---|---|---|
| $HgCl_2$ | 98 ± 7 | Total extractable, 92 ± 9 |
| $CH_3HgCl$ | 92 ± 17 | |
| $C_2H_5HgCl$ | 92 ± 17 | |

*95% confidence level, n=3

Data obtained from Ref. 6.
These data are provided for guidance purposes only.

TABLE 4

RECOVERIES OF EXTRACTABLE MERCURY SPECIES SPIKED
INTO NATURAL SOIL MATRIX 1

| Mercury spiked species | Recovery* (%) HPLC-ICP-MS | Recovery* (%) Method 7473 |
|---|---|---|
| $HgCl_2$ | 106 ± 15 | 92.5 $\pm$ 0.5 |
| $CH_3HgCl$ | 104 ± 10 | 95.5 $\pm$ 2.5 |
| $C_2H_5HgCl$ | 92 ± 18 | 91.0 $\pm$ 3.0 |
| HgO | NA | 93.0 $\pm$ 1.0 |

*95% confidence interval with n = 3

The soil matrix 1 was a soil collected from waste deposit field and sieved through 60-mesh screen, then heated in a 240 °C oven for one week. The mercury residue in the soil matrix was less than 30 ng mercury per g of soil.

Data obtained from Ref. 6.
These data are provided for guidance purposes only.

TABLE 5

RECOVERIES OF EXTRACTABLE MERCURY SPECIES SPIKED
INTO NATURAL SOIL MATRIX 2

| Mercury spiked species | Recovery* (%) HPLC-ICP-MS | Recovery* (%) Method 7473 |
|---|---|---|
| $HgCl_2$ | 12 ± 6 | Total extractable, 62 ± 8 |
| $CH_3HgCl$ | 89 ± 12 | |
| $C_2H_5HgCl$ | 95 ± 15 | |

*95% confidence interval, n=3
The low recovery of the $HgCl_2$ is probably due to complexing of the $Hg^{2+}$ with components of the sample matrix.Data obtained from Ref. 6.

These data are provided for guidance purposes only.

TABLE 6

COMPARISON OF 2.0%HCl+10% ETHANOL EXTRACTION WITH METHOD 1311 (TCLP) FOR EXTRACTABLE MERCURY SPECIES

| Reference Material | 2.0% HCl + 10% Ethanol extraction[a] | Method 1311[b] |
|---|---|---|
| BCR 580 | 1.4 ± 0.4 ppm | 3 ± 2 ppb |
| SRM 2709 | 40 ± 8 ppb | 12 ± 9 ppb |

[a]95% confidence interval, n=3.

[b]Method 1311 was scaled down to a 1-gram sample size

Data obtained from Ref. 6. These data are provided for guidance purposes only.

TABLE 7

RECOVERIES OF EXTRACTABLE MERCURY SPECIES AFTER SCF SOLID-PHASE EXTRACTION

| Eluent | $CH_3HgCl$ | $C_2H_5HgCl$ | $HgCl_2$ |
|---|---|---|---|
| Pass-through (10% Ethanol) | <0.5% | <0.5 | <0.5% |
| 1.0 M HCl + 1.0 M NaCl | 96% | 99% | <0.1% |
| 6 M HCl + saturated NaCl + 0.1% $CuCl_2 \cdot 2H_2O$ | <3% | <3% | 98% |
| Residue (in SCF) | <DL | <DL | <DL |

DL = detection limit (0.01 ng)

Data obtained from Ref. 6.
These data are provided for guidance purposes only.

TABLE 8

VALIDATION OF THE METHOD USING BCR 580

| Method or (Certified Value) | Methyl Mercury as Mercury (ppb) | Extractable Inorganic Mercury (ppm) | Non-extractable Mercury (ppm) | Total Mercury (ppm) |
|---|---|---|---|---|
| HPLC-ICP-MS | 73.6 ± 6.3[a] | 1.4 ± 0.4[b] | 133 ± 9.6[b] | 134.5 ± 9.6[c] |
| SCF-Method 7473 | 78 ± 27[b] | 0.9 ± 0.3[b] | 127 ± 7[b] | 128 ± 7[c] |
| (Certified Value) | 70.2 ± 3.4 | NA | NA | 132 ± 3 |

[a]95% confidence interval with n = 6

[b]95% confidence interval with n = 3

[c]95% confidence interval with n = 3

NA = not analyzed

Data obtained from Ref. 6.
These data are provided for guidance purposes only.

TABLE 9

VALIDATION OF MICROWAVE-ASSISTED EXTRACTION METHOD

| Sample | Certified / 'Made-to' Value (µg/g) | | Method 7473 (direct analysis) (µg/g) | HPLC-ICP-MS (µg/g) | | |
|---|---|---|---|---|---|---|
| | $Hg^{2+}$ | $CH_3Hg^+$ | Total | $Hg^{2+}$ | $CH_3Hg^+$ | Total |
| ***Material-1*** | | | | | | |
| Inorganic Mercury | 4.0 | ---- | 4.08 ± 0.16 | 4.26 ± 0.17 | ND | 4.26 ± 0.17 |
| Organic Mercury | ---- | 4.0 | 3.58 ± 0.27 | ND | 3.81 ± 0.20 | 3.81 ± 0.20 |
| Mixed Mercury | 3.0 | 3.0 | 5.73 ± 0.58 | 3.02 ± 0.06 | 2.66 ± 0.07 | 5.68 ± 0.09 |
| ***Material-2*** | | | | | | |
| Inorganic Mercury | 6.0 | ---- | 6.73 ± 1.04 | 6.06 ± 0.56 | ND | 6.06 ± 0.56 |
| Organic Mercury | ---- | 6.0 | 5.44 ± 0.62 | ND | 5.94 ± 0.52 | 5.94 ± 0.52 |
| ***Standard Reference Materials*** | | | | | | |
| SRM 1941a | 0.5 ± 0.2 | ---- | 0.61 ± 0.02 | 0.67 ± 0.06 | ND | 0.67 ± 0.06 |
| SRM 2704 | 1.44 ± 0.07 | ---- | 1.51 ± 0.05 | 1.40 ± .08 | ND | 1.40 ± 0.08 |
| SRM 2709 | 1.40 ± 0.08 | ---- | 1.46 ± 0.03 | 1.28 ± 0.12 | ND | 1.28 ± 0.12 |

ND = not detectable (ND = 0.05 ng)
Uncertainties are expressed at the 95% confidence level with n = 3.
Material-1: 100% processed topsoil; and Material-2: a mixture of 75% processed topsoil and 25% Ottawa sand

Data obtained from Ref. 16, 17.
These data are provided for guidance purposes only.

TABLE 10

THE DECONVOLUTED CONCENTRATION AND TRANSFORMATION OF MERCURY SPECIES IN MATERIAL-1 USING SIDMS CALCULATIONS.

| | Deconvoluted Concentration | | Interconversion | |
|---|---|---|---|---|
| | $Hg^{2+}$ (µg/g) | $CH_3Hg^+$ (µg/g) | $Hg^{2+}$ to $CH_3Hg^+$ (%) | $CH_3Hg^+$ to $Hg^{2+}$ (%) |
| DSBE | 3.05 ± 0.12 | 2.69 ± 0.10 | 1.3 ± 1.5 | 0.1 ± 1.4 |
| DSAE | 2.94 ± 0.07 | 2.62 ± 0.09 | 0.8 ± 1.5 | 0.7 ± 0.6 |

DSBE = double spiked before extraction. DSAE = double spiked after extraction.
Uncertainties are expressed at the 95% confidence level with n = 3

Data obtained from Ref. 16, 17.
These data are provided for guidance purposes only.

TABLE 11
SIDMS ANALYSIS (6800) OF THREE EXTRACTABLE MERCURY SPECIES AND NON-EXTRACTABLE MERCURY FROM CRUDE OIL AFTER EPA METHOD 3200 EXTRACTION

| Sample Name | Extractable Mercury Species, µg/kg | | | Non-Extractable Mercury Species, µg/kg | Total Mercury*, µg/kg |
|---|---|---|---|---|---|
| | $Hg^{2+}$ | $MeHg^{+}$ | $EtHg^{+}$ | | |
| Serial # 871833 | 17.3 ± 3.6 | 0.8 ± 0.1 | 10.7 ± 1.1 | 342.5 ± 46.8 | 370.2 ± 47.0 (414.1 ± 24.7) |
| Serial # 870987 | 23.6 ± 3.7 | 0.5 ± 0.1 | 7.4 ± 2.2 | 323.3 ± 30.7 | 354.8 ± 31.0 (437.8 ± 25.4) |

Uncertainties are at 95% CL, n = 12 (3 x 4).

*values in parentheses are total mercury determined by isotope dilution mass spectrometry (IDMS) of the crude oil after Method 3052 digestion.

Data obtained from Ref. 26.
These data are provided for guidance purposes only.

TABLE 12
MERCURY SPECIATION IN HUMAN HAIR CERTIFIED REFERENCE MATERIAL, IAEA-085, BY HPLC-ICP-MS AND QUANTIFICATION BY EXTERNAL CALIBRATION.[a] THE VALUES ARE MEANS ± 95% CL (n = 4 x 4)[b]

| Extraction procedure | $Hg^{2+}$ (as Hg) (µg/g) | $CH_3Hg^{+}$ (as Hg) (µg/g) | Sum of Species (µg/g) | Mean degree of Transformation (%) | |
|---|---|---|---|---|---|
| | | | | $Hg^{2+}$ to $CH_3Hg^{+}$ | $CH_3Hg^{+}$ to $Hg^{2+}$ |
| Method 3200 (ultrasonication) | 0.33 ± 0.06 | 20.87 ± 0.37 (91 ± 2) | 21.21 ± 0.38 (91 ± 2) | ----- | ----- |
| Method 3200 (ultrasonication) and Method 6800 | 1.13 ± 0.25 | 19.80 ± 1.25 (87 ± 6) | 20.93 ± 1.28 (90 ± 6) | 9 ± 3 | 0 ± 1 |
| Method 3200 (MAE) | 1.80 ± 0.61 | 22.67 ± 1.54 (99 ± 7) | 24.67 ± 1.65 (106 ± 7) | ----- | ----- |
| Method 3200 (MAE) and Method 6800 | 0.59 ± 0.22 | 23.65 ± 1.42 (103 ± 6) | 24.24 ± 1.44 (105 ± 6) | 4 ± 2 | 6 ± 1 |

[a]Certified total Hg is = 23.2 ± 0.8 µg/g, certified $CH_3Hg^{+}$ is = 22.9 ± 1.0 µg/g and estimated $Hg^{2+}$ is 0.1 – 0.5 µg/g. [b]The percent recoveries of total Hg and $CH_3Hg^{+}$ are indicated in parentheses.

Data obtained from Ref. 27.
These data are provided for guidance purposes only.

TABLE 13
MERCURY SPECIATION IN TUNA FISH CERTIFIED REFERENCE MATERIAL (ERM-CE-464) BY METHOD 6800 HPLC-ICP-MS AFTER EXTRACTION WITH METHOD 3200 WITH AND WITHOUT SPIKING ISOTOPICALLY ENRICHED ANALOGUES.

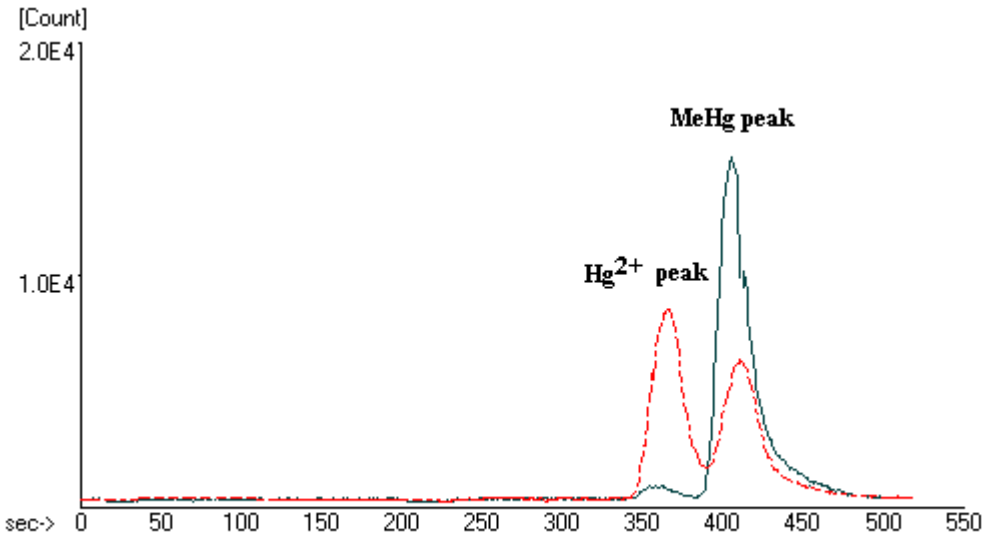
| | $Hg^{2+}$ (mg/kg) | $CH_3Hg^+$ (mg/kg) | Sum of Species (mg/kg) | Degree of transformation, mean | |
|---|---|---|---|---|---|
| | | | | $Hg^{2+}$ to $CH_3Hg^+$ | $CH_3Hg^+$ to $Hg^{2+}$ |
| Method 3200 | 0.06 ± 0.04 | 3.94 ± 0.12 (77 ± 2) | 4.00 ± 0.13 (76 ± 2) | ------ | ------ |
| Method 3200 and Method 6800 | 0.11 ± 0.07 | 5.60 ± 0.33 (109 ± 6) | 5.71 ± 0.34 (109 ± 6) | 18 ± 4 | 0.8 ± 0.6 |

Certified total Hg is 5.24 ± 0.10 mg/kg, certified $CH_3Hg^+$ is 5.12 ± 0.16 mg/kg and estimated $Hg^{2+}$ is 0.12 mg/kg. The percent recoveries of total Hg and $CH_3Hg^+$ are indicated in parentheses.
Isotopic spiking as described in Method 6800 prior to extraction with Method 3200

Data obtained from Ref.28.
These data are provided for guidance purposes only.

FIGURE 1

METHYL MERCURY TRANSFORMATION

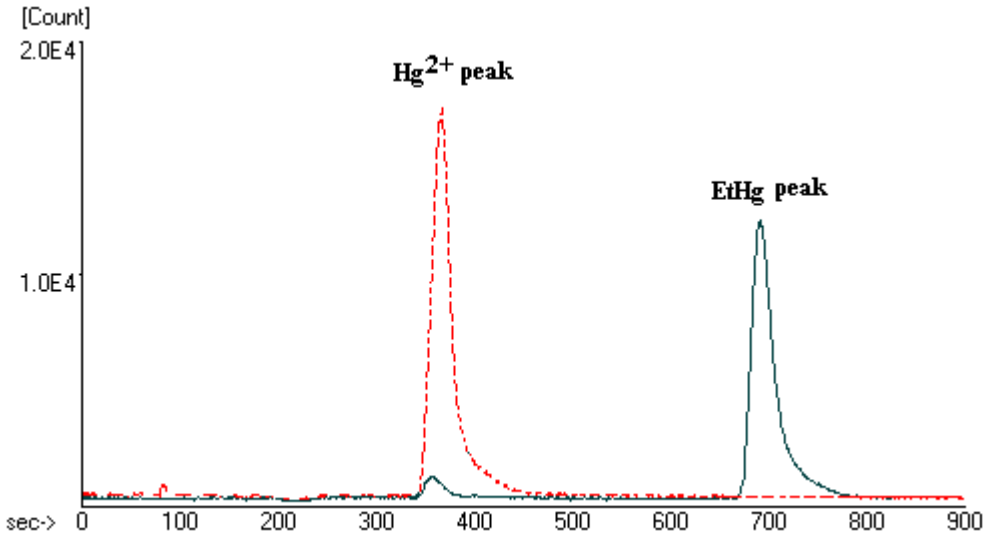



Solid line: A methyl mercury standard in acid solution before the addition of iron
Dash line: A methyl mercury standard in acid solution with 500 ppm iron.

NOTE: Transformation observed 8 hr after spiking (HPLC flow rate 0.8 mL/min).

Figure taken from Ref. 6.
These data are provided for guidance purposes only.

FIGURE 2

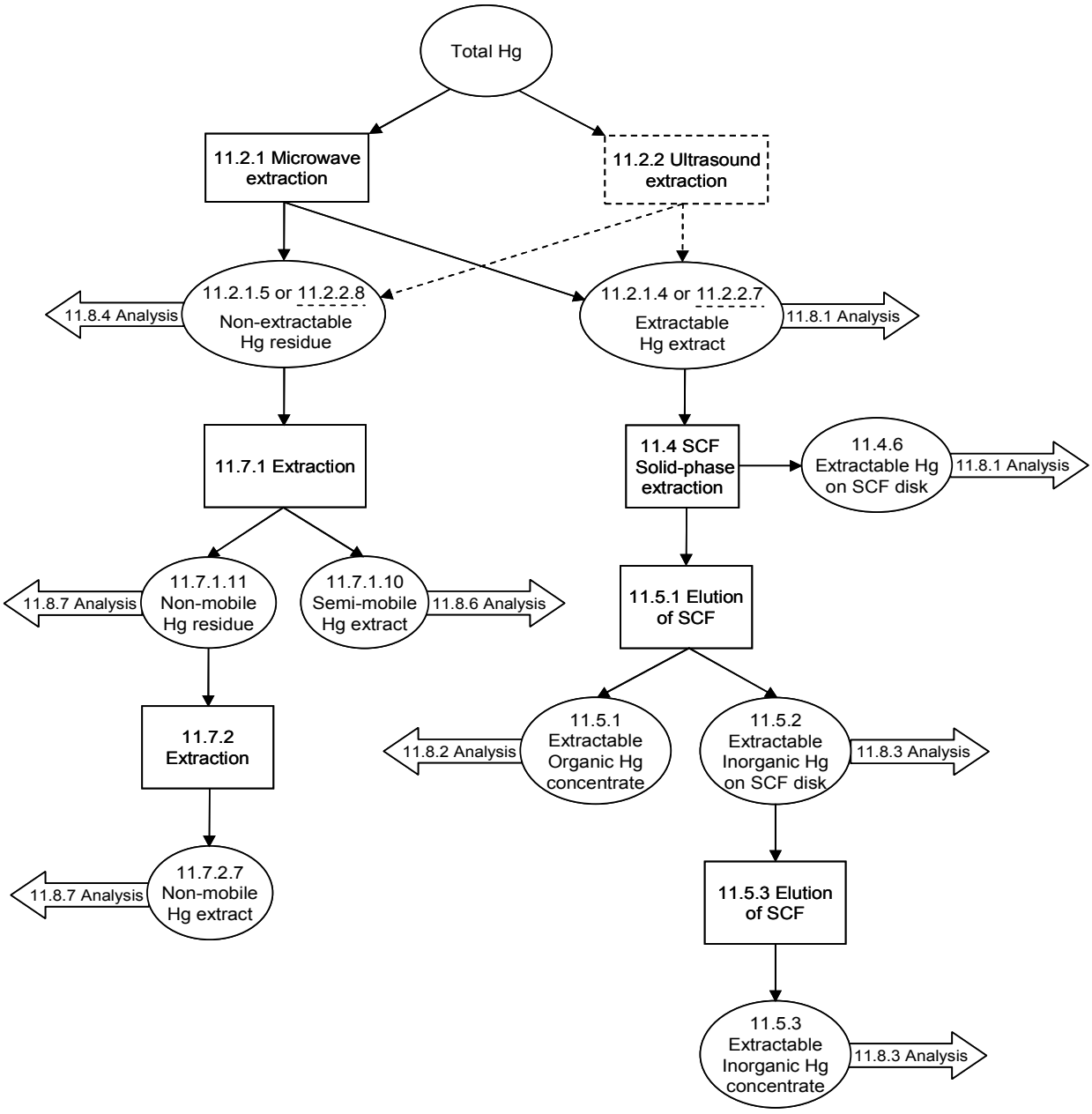



Solid line: An ethyl mercury standard in acid solution without the addition of iron
Dash line: An ethyl mercury standard in acid solution with 500 ppm iron.

NOTE: Transformation observed 8 hr after spiking (HPLC flow rate 0.8 mL/min).

Figure taken from Ref. 6.
These data are provided for guidance purposes only.

## MERCURY SPECIES FRACTIONATION AND QUANTIFICATION BY MICROWAVE ASSISTED EXTRACTION, SELECTIVE SOLVENT EXTRACTION AND/OR SOLID PHASE EXTRACTION



Appendix A:

Summary of Revisions to Method 3200 (as compared to previous Revision 0, July 2005)

1. Improved overall method formatting for consistency with new SW-846 methods style guidance. The revision number was changed to one and the date published to July 2014.
2. Updated and expanded “QUALITY CONTROL” section for better adherence to current SW-846 method guidelines and for improved alignment with current universal practices for published analytical methods.
3. Additional comment added in Sec. 8.3 concerning holding times.
4. Minor editorial and technical revisions were made throughout to improve method clarity.